MHC Class II induction in oligodendroglia varies with age

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Introduction

- Oligodendroglia have been shown to upregulate immune proteins including major histocompatibility complex (MHC) after exposure to IFNγ and in mouse models of multiple sclerosis (MS)[1, 2]
- MS progression correlates with age[3] and recent evidence suggests that immune properties of oligodendroglia similarly vary across the lifespan[4,5,6]
- We previously developed B2M-tdTomato and CD74-tdTomato mice to identify MHC class I and class II-expressing cells *in vivo,* respectively, and characterized MHC-expressing oligodendroglia in the central nervous system (CNS) of MS mouse models in young adult mice [7]
- We hypothesized that MHC induction in oligodendroglia is age-dependent and leveraged our MHC reporter lines to determine how neonatal (p7-p9), young adult (10-16 weeks), and aged adult (52 weeks) oligodendroglia differ under inflammatory conditions

Methods

- Immunohistochemistry and immunocytochemistry: Sections and cells were permeabilized in PBS + Triton (PBST), blocked for 1 hour in PBST + serum at room temperature, and incubated overnight at 4 degrees in primary antibodies in PBST with serum. They were incubated in secondary antibodies in PBST + serum for 1 hour at room temperature.
- Image quantification: Images were quantified by hand in Zen Blue while total DAPI+ cell counts were done using FIJI.
- Oligodendroglia isolation: Forebrains were dissociated using papain with DNAse. O4+ cells were isolated using Miltenyi's O4+ kit. For PDGFRα+ OPCs, immunopanning was done using a BSL-1 plate to remove endothelial cells, CD11b plate to remove microglia, and PDGFRα plate to positively select for OPCs. Once cells reached confluency, they were treated with 50 ng/mL mIFNγ for 72 hours and then collected for qPCR or ICC.
- qPCR: RNA was isolated using RNeasy Plus Micro kit. First-strand cDNA was synthesized using iSCRIPT cDNA Synthesis. qPCR was conducted using iQ SYBR. Delta delta Ct analysis was conducted by normalizing to beta actin.

MHC class II-expressing oligodendroglia are found in the spinal cord of 52-week-old mice

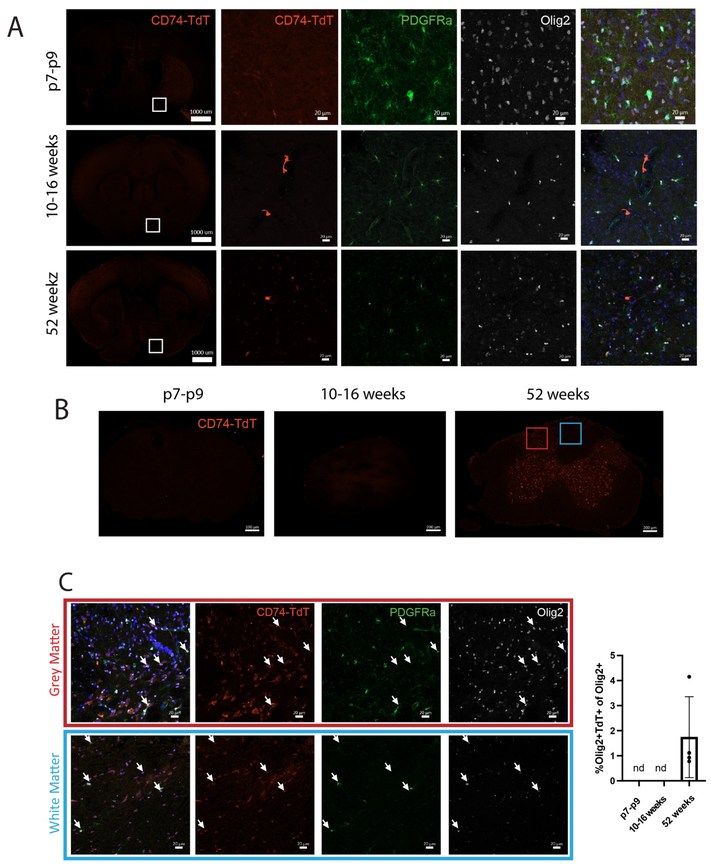


Fig 1 – CD74-TdT+Olig2+ cells in the CNS of p7-p9, 10-16, and 52-week-old mice. Representative images of brain immunohistochemistry from CD74-TdT mice. White squares indicate areas selected for higher magnification. (A) Representative images of TdT signal in lumbar spinal cord sections from CD74-TdT mice. Red and blue squares on aged spinal cord indicate areas selected for higher magnification. (B) Representative images and quantification of Olig2 and TdT colocalization in lumbar spinal cord sections from CD74-TdT mice. White arrowheads indicate colocalization between Olig2 and CD74-TdT. N=3-4 animals per age group, 3-4 whole sections per animal. Data represented as mean ± standard deviation. (C)

MHC class I-expressing oligodendroglia are present in the CNS across age

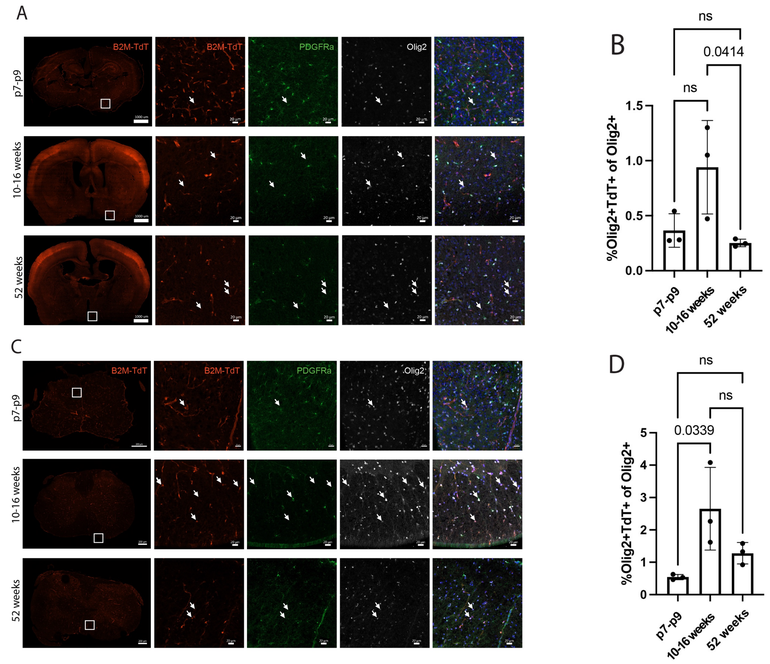


Fig 2 – B2M-TdT+Olig2+ cells in the CNS of p7-p9, 10-16, and 52-week-old mice. Representative images of TdT signal in brain sections from B2M-TdT mice. White squares indicate areas selected for higher magnification. White arrowheads indicate colocalization between Olig2 and B2M-TdT. (A) Quantification of Olig2 and TdT colocalization in the ventral brain of B2M-TdT mice. N=3 animals per age group, 3-4 sections per animal, 6 ventral regions per section (based on localization of cells of interest in pilots). One-way ANOVA. (B) Representative images of TdT signal in lumbar spinal cord sections from B2M-TdT mice. White squares indicate areas selected for higher magnification. (C) Quantification of Olig2 and TdT colocalization in the lumbar spinal cord of B2M-TdT mice. N=3 animals per age group, 3-4 whole sections per animal. One-way ANOVA. All data represented as mean ± standard deviation. (D)

p7-p9 O4+ cells upregulate more MHC class II than 8-12 week cells after IFNγ exposure

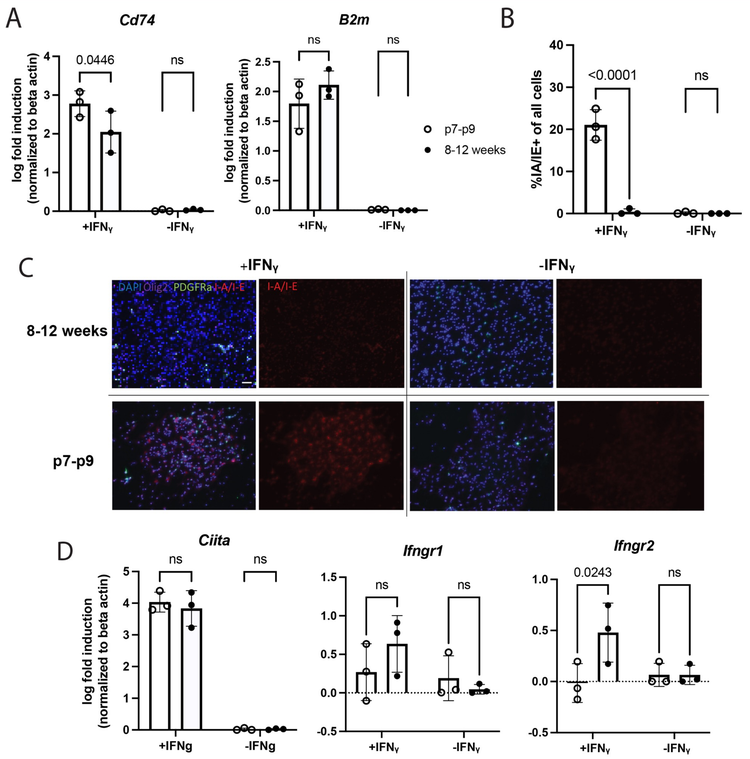
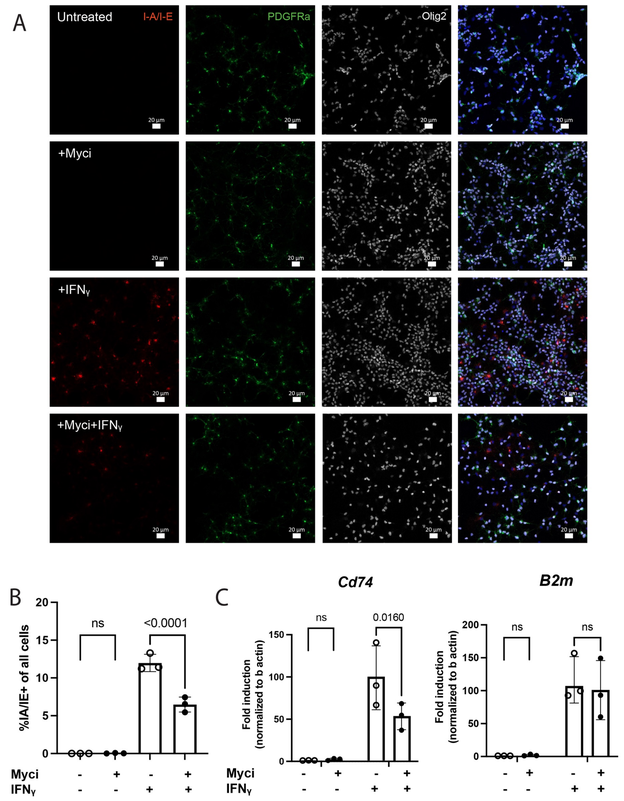


Fig 3 – Reduced MHC class II induction in 8–12-week-old oligodendroglia is not due to a general deficit in IFNγ response. Fold induction of *Cd74* and *B2m* by qPCR in O4+ cells. N=3 biological replicates per age group. Two-way ANOVA. (A) Quantification of I-A/I-E (MHC class II marker) immunocytochemistry of IFNγ-treated O4+ cells. N=3 biological replicates per age group, 2000-10772 cells quantified per condition replicate. Two-way ANOVA. (B) Representative images of I-A/I-E immunocytochemistry of IFNγ-treated O4+ cells. (C) Fold induction of *Ifngr1, Ifngr2* and *Ciita* by qPCR in O4+ cells. N=3 biological replicates per age group. Two-way ANOVA. All data represented as mean ± standard deviation. (D)

Inducing an aged phenotype in OPCs reduces MHC class II induction



Myci reduces MHC class II, but not class I, induction in neonatal OPCs. Myci, an inhibitor of c-MYC, has previously been shown to induce an aged phenotype in neonatal OPCs. Representative images of I-A/I-E staining in neonatal OPC cultures treated with Myci, OPCs[8] and IFNγ. (A) Quantification of I-A/I-E staining in OPC cultures treated with Myci and IFNγ. N=3 biological replicates per age group, 3912-7081 cells quantified per condition replicate. Two-way ANOVA. (B) Fold induction of *Cd74* and *B2m* by qPCR in OPCs treated with or without Myci. N=3 biological replicates per age group. Two-way ANOVA. All data represented as mean ± standard deviation. (C)

Conclusions

- In naïve mice, MHC class II-expressing oligodendroglia are specific to the 52-week-old spinal cord while MHC class I-expressing cells are present in the brain and cord across ages.
- When exposed to IFNγ *in vitro,* O4+ cells from 8-12-week-old mice upregulate less MHC class II than p7-p9 cells, but MHC class I is unaffected, suggesting a specific deficit in MHC class II regulation rather than IFNγ response broadly.
- P7-p9 and 8-12-week-old O4+ cells express similar levels of the machinery needed to respond to IFNγ and upregulate MHC class II.
- Myci, previously shown to induce an aged phenotype in neonatal OPCs, suppresses OPC induction of MHC class II, but not class I, in response to IFNγ.
- *In vivo* studies of oligodendroglia response to inflammation across age are needed to validate these observations, as are investigations into the mechanisms underlying these age differences in MHC class II induction.

References

1 Kirby et al, *Nature Communications*, 2019 2 Falcao et al, *Nature,* 2018 3 Graves et al, *Lancet,* 2023 4 Yaquibi et al, *Glia,* 2022 5 Spitzer et al, *Neuron,* 2019 6 de la Fuente et al., *Cellular and Molecular Proteomics,* 2020 7 Harrington and Catenacci et al, *eLife,* 2023 8 Neumann et al, *Nature Aging,* 2021

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